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14. ABSTRACT During the next funding period we intend to address four major questions. 1) How does JP-8 induce immune suppression? Does JP-8 alter immune responsiveness through its effect on cytokine production or does it alter the ability of antigen presenting cells to present antigen? 2) Does JP-8 exposure affect ongoing immune reactions? Will exposure to JP-8 interfere with the immune response to recall antigens and block the protective effect of prior immunization? 3) How can we prevent JP-8 induced immune suppression? Once a better understanding of the suppressive mechanism is obtained, we will begin to design rational steps aimed at preventing its immune suppressive effect. 4) What components in JP-8 induce dermal immunotoxicity? JP-8 is produced from commercial Jet-A by use of an additive package containing diethylene glycol monomethyl ether, (DiEGME) an anti-icing compound. Because some have suggested DiEGME is a dermal immunotoxin we will concentrate on determining if application of DiEGME induces immune suppression. The specific aims of this proposal are designed to gain an understanding of the mechanism(s) involved in JP-8-induced immune suppression. The long-term of this work is to use the knowledge obtained here to design a procedure to prevent JP-8-induced immune suppression and limit its potential harmful effect on USAF personnel.					
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Final AFOSR Progress Report

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- 2. Title of Proposal:** Immune Suppression by Dermal Exposure to JP-8 jet fuel
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- 4. Name of Institution:** The University of Texas, M.D. Anderson Cancer Center,
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6. List of Manuscripts submitted/published:

- Ramos, G., Nghiem, D.X., Walterscheid, J.P., and Ullrich, S.E. Dermal application of jet fuel suppresses secondary immune reactions. *Toxicology and Applied Pharmacology* 180:136-144, 2002.
- Ramos, G., Kazimi, N., Nghiem, D.X., Walterscheid, J.P., and Ullrich, S.E. Platelet activating factor receptor binding plays a critical role in jet fuel-induced immune suppression. *Toxicology and Applied Pharmacology* 195:331-338, 2004
- Vijayalaxmi, Kligerman, A.D., Prihoda, T.J. and Ullrich, S.E. Cytogenetic studies in mice treated with the jet fuels, Jet-A and JP-8. *Cytogenetic and Genomic Research* 104:371-375, 2004.
- Ullrich, S.E. Mechanism by which Ultraviolet radiation, a ubiquitous environmental agent, suppresses the immune response. In: *Immunotoxicology and Immunopharmacology*, 3rd edition, R Luebke, ed., CRC Press, Boca Raton FL. *In press (although the major focus of this paper regards the immunosuppressive effects of UV, the discussion deals with the mechanisms common to UV and jet fuel-induced immune suppression, as such it should be of interest to immunotoxicologists and the AFOSR)*

7. Scientific personnel supported by this grant:

Stephen E. Ullrich, PI
Nasser Kazimi, Senior Research Associate
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8. Inventions/Patents/Discoveries None

9. Collaborators/Consultants: Vijayalaxmi, Department of Radiation Oncology, The University of Texas, Health Science Center, San Antonio, Texas.

Dr Vijayalaxmi and I are testing the hypothesis that dermal treatment with jet fuel induces genotoxic damage to blood and bone marrow cells. We recently reported exposure to JP-8 and Jet-A induces the formation of micronuclei in polychromatic erythrocytes and bone marrow cells. The studies were done in a blinded fashion; the mice were treated in Houston, blood and bone marrows smears were sent the slides to San Antonio for analysis.

10. Honors and Awards: A. During the past year I was appointed as the first recipient of the Dallas/Fort Worth "Living Legends" Professorship in Cancer Research at MD Anderson Cancer Center.

B. I was recently elected the President of the American Society for Photobiology. I will serve as President-elect for one year and then serve a one-year term as President from July 2006 to July 2007.

11. Key Findings/Results/Accomplishments:

The focus of the proposal was to address 4 basic questions:

1. How does dermal jet fuel exposure induce immune suppression?
2. Can we prevent JP-8-induced immune suppression in exposed individuals?
3. Will dermal exposure to jet fuel suppress secondary immune reactions?
4. What are the components in JP-8 that are responsible for inducing immune suppression?

We made significant progress in all areas by providing answers for all the questions posed above.

Questions 1 & 2: How does JP-8 induce immune suppression and how can we use this information to design therapies to block JP-8-induced immune suppression in exposed personnel? Understanding the mechanism by which JP-8 induces immune suppression is important for a number of reasons. First, it satisfies our basic intellectual curiosity and helps to move the field of immunotoxicology forward. No other laboratory in the world studying jet fuel-induced toxicology has made more contributions to further our understanding of the mechanism(s) by which jet fuel exposure induces immune suppression. Second, it is clear that JP-8 will become the universal military fuel of the future and it is equally clear that risk avoidance by military personnel is next to impossible. There is a critical need to develop therapies to reverse the immunosuppressive effects of jet fuel exposure. In this respect, we have

made a number of significant contributions. During our initial funding period (Mechanisms responsible for immune suppression following acute exposure to volatile organic chemicals) we discovered that JP-8 treatment preferentially suppresses cell-mediated immune reactions (delayed and contact hypersensitivity and T cell proliferation) but not antibody formation by inducing the release of biologic response modifiers such as prostaglandin E₂ and interleukin (IL)-10. Moreover, we demonstrated that blocking the biological activity of IL-10 with monoclonal antibodies or recombinant IL-12 would reverse jet fuel induced immune suppression. In addition, blocking prostaglandin E₂ secretion, with a selective cyclooxygenase-2 inhibitor, also overcame jet fuel-induced immune suppression. These results were published in two papers appearing in *Toxicological Sciences* (52:61, 1999; and 58:290, 2000).

The activation of the cyclooxygenase (COX) enzyme is the rate-limiting step in the production of prostaglandin E₂. Its activity can be blocked by a number of common pharmaceuticals (aspirin, non-steroidal anti-inflammatory drugs, such as ibuprofen, and selective COX-2 inhibitors). Studies by others have indicated that skin damage in response to stress, UV radiation, and chemical insult causes the release of the lipid mediator of inflammation, platelet activating factor (PAF), which activates the transcription of a number of genes including COX-2 and IL-10. Therefore, we tested the hypothesis that COX-2 activation in jet fuel-treated mice was secondary to the production of PAF. We blocked the downstream effects of PAF by using a series of selective PAF receptor antagonists. We found that JP-8-induced immune suppression was totally abrogated by use of these selective antagonists, a class of drugs that have been shown to be safe in humans. Because one prominent way of inducing PAF in vivo is via oxidative stress, we next tested the hypothesis that anti-oxidants, such as beta-hydroxy toluene (BHT), Vitamin C and Vitamin E, would reverse JP-8-induced immune suppression. Administration of all three after JP-8-treatment totally reversed immune suppression. These studies were described in the paper published in *Toxicology and Applied Pharmacology* in 2004.

The net result of our mechanistically driven studies has been the development of at least 5 different classes of drugs/therapies that block JP-8-induced immune suppression (anti-IL-10, recombinant IL-12, cyclooxygenase inhibitors, PAF-receptor antagonists and anti-oxidants). Some are clearly not applicable to humans because of the cost (monoclonal anti-IL-10) or toxicity (recombinant IL-12 and perhaps chronic use of the selective COX-2 inhibitors). Others, however, such as common anti-oxidants (Vitamin C), PAF-receptor antagonists and some cyclooxygenase inhibitors (aspirin, non-steroidal anti-inflammatory drugs) may provide promising, low cost and safe methods to ameliorate JP-8-induced immune suppression in exposed individuals. These findings are our major contribution to the field.

Question #3: Does JP-8 treatment suppress ongoing immune reactions: At first glance this question may appear to be of little interest to the non-immunologist. We would argue, however, that the answer to this question is of great importance to the military community. Perhaps the most important medical advance of the twentieth century was the reduction, and in some cases the eradication (i.e., smallpox) of morbidity and mortality due to infectious disease by widespread use of vaccination. If dermal exposure to JP-8, suppresses secondary immune reactions, such as immunological memory, it may suggest that protection against infectious disease could be compromised in exposed individuals. This could be especially critical during military operations where immunization (i.e., anthrax) is used to protect combat personnel from the threat of biological weapons. Using a mouse model, we found that JP-8 treatment suppressed the immune response in mice previously immunized with the opportunistic fungal antigen *Candida albicans*. In addition, jet fuel treatment suppressed immunological memory.

Fortunately, the immune suppression was overcome by treating the mice with a selective COX-2 inhibitor. This suggests that the mechanisms involved in JP-8-induced suppression of primary and secondary immune reactions is the same, COX-2 activation is involved, and blocking the activation of COX-2, and the production of prostaglandin E₂ will protect exposed individuals. These findings were published in *Toxicology and Applied Pharmacology*, 2002.

4. What components in JP-8 induce immune suppression? JP-8 is essentially commercial Jet-A with three additives, anti-freeze (diethylene mono-methyl ether), an anti-corrosive agent (DCI-4A), and an anti-static reagent (STATIS-450). It is not clear whether the immune suppressive properties of JP-8 are inherent to the base fuel or a consequence of one of more of the additives a refinery blends into Jet-A to produce JP-8. To address this question we first asked if Jet-A was immune suppressive. In all of the studies mentioned above, we set up parallel experiments, using JP-8 and using equal concentrations of Jet-A. We found the following: A) Jet A suppresses both primary and secondary immune reactions. B) The concentrations needed to induce immune suppression were the same in that the dose response curves were identical. C) We found that PAF-receptor antagonists, selective COX-2 inhibitors, and anti-oxidant treatment all blocked Jet-A induced immune suppression. The concentrations required to block Jet-A-induced immune suppression were identical to those needed to block JP-8-induced immune suppression. These data indicate that the additive package is not responsible for the induction of immune suppression, but rather the induction of immune suppression is inherent to the base kerosene fuel. They also imply that the mechanisms by which JP-8 and Jet-A induce immune suppression are identical. These studies were described in the two papers published in *Toxicology and Applied Pharmacology*, 2002 and 2004.

12. Technology Transfers: None